

STUDIES ON THE CILIATES FROM FRESH WATER MUSSELS

I. THE STRUCTURE AND NEUROMOTOR SYSTEM OF CONCHOPHTHIRIUS ANODONTÆ STEIN, C. CURTUS ENGL., AND C. MAGNA SP. NOV.

GEORGE W. KIDDER

(From the College of the City of New York and the Marine Biological Laboratory,
Woods Hole, Mass.)

Although the ciliate commensals of the various fresh water mussels exist in great abundance and offer excellent material for a cytological study, very little attention has been paid to them. The greatest number of these organisms belong to the genus *Conchophthirius*. Indeed, the type species *C. anodontæ* (Ehr.) Stein is found in one of these mussels. Since the work of Schuberg in 1889 there has been but one paper of merit,—that of Raabe (1933),—dealing extensively with this genus, and no work, as far as I am aware, of a strictly cytological nature. Raabe has given an excellent review of the literature and an account of certain morphological features of four species of *Conchophthirius*. Employing the silver nitrate method of Klein (1926*a, b*), he was able to establish the existence of a previously undescribed species, *C. unionis*, and to place the ciliate called by Claparède and Lachmann (1858) *Plagiotoma acuminata* in the genus *Conchophthirius*. He has based his specific comparisons chiefly on the pattern of the “silverline system” and only noted those extra characteristics which were easily observed in life. Schuberg (1889), while giving an excellent description of *Conchophthirius anodontæ* and *C. steenstrupii*, did not recognize Engelmann’s (1862) *C. curtus*, but it is quite obvious, if one is to observe even cursorily the forms from various species of *Anodonta*, that in this judgment Schuberg was wrong.

To summarize briefly the existing species of the genus *Conchophthirius* so far known to the literature, I shall list only those which I consider valid. For a more extensive discussion the reader is referred to the work of Raabe (1933). The list is as follows: *Conchophthirius anodontæ* (Ehr.) Stein 1861, *C. steenstrupii* Stein 1861, *C. (Plagiotoma) acuminata* Claparède and Lachmann 1858, *C. curtus* Engelmann 1862, *C. antedonis* André 1910, *C. discophorus* Mermod 1914, *C. mytili* deMorgan 1925, *C. unionis* Raabe 1933, and *C. caryoclada* Kidder 1933*a*.

Attention should be directed to the ciliate called *Conchophthirius metchnikoffi* by Certes (1891), which was later removed to the genus *Phascodinium*, order Heterotrichida, and to *Conchophthirius elongatus* and *C. lamellidens* of Ghosh (1918). The last two species cannot be considered seriously, as the descriptions and illustrations of Ghosh are far too brief and sketchy to allow one to judge their validity.

It is the purpose of this paper to present the results of a cytological investigation of the structure and neuromotor apparatus of members of the genus *Conchophthirius* encountered in the examination of seven species of mussels taken from fresh water lakes in Massachusetts and New York. It is my hope that this work will lead to a clearer understanding of the relationships which exist between the external fibrillar system, as described so carefully by Raabe (1933), and the internal fibrillar system demonstrated by the methods here employed.

The species with which I shall deal are *Conchophthirius anodonta* (Ehr.) Stein, *C. curtus* Engl. and a hitherto undescribed species, which, because of its large size, I shall call *Conchophthirius magna*.

The basic structural differences between these species will be pointed out in the general descriptions. Because of the very close agreement of their neuromotor systems, these elements in all three species will be considered together, attention being directed only to points of difference.

This investigation was carried on at the Marine Biological Laboratory at Woods Hole, Massachusetts. I wish to express my indebtedness to W. J. Clench, Curator of Molluscs in the Museum of Comparative Zoology of Harvard University, who very kindly identified the species of mussels used in this work.

MATERIAL AND METHODS

The three species of ciliates to be described are found in the fluids of the mantle cavities and creeping about over the gills and palps of various species of fresh water mussels. The infections vary from only a few ciliates per mollusc in the cases of *Anodonta implicata*, *A. cataracta*, *Lampsilis radiata*, *L. cariosa* and *Alasmidonta undulata* to hundreds in the cases of *Anodonta marginata* and *Elliptio complanatus*.

In an attempt to determine the exact location of the ciliates in their hosts many mussels were opened carefully and parts of the mantle, gills, and palps dissected out and examined. In the cases of *C. curtus* and *C. magna* no definite location seemed to be preferred as ciliates were found on all of the exposed surfaces and also swimming freely in the mantle fluids. But in the case of *C. anodonta* there invariably occurred a localization of organisms on the non-ciliated surface of the palps. The ciliates found here exhibited entirely different reactions in regard to movement from those found elsewhere. When the palp was removed

from the mollusc and carefully flattened out in a Syracuse dish, the non-ciliated surface appeared mottled with *C. anodontæ*. In contrast to the ceaseless activity normally encountered, they remained extremely quiet, attached by the cilia of their ventral surfaces. Examination under high magnifications showed the cilia of the peristomal region alone in vigorous motion while the body cilia waved listlessly. When dislodged from the palp the organisms moved rapidly and continued this activity until they became moribund.

There appears to be a fair degree of host specificity in this group. *Conchophthirius magna* was found only in *Elliptio complanatus* and was always accompanied by *C. anodontæ*, while *C. curtus* was found in *Anodonta marginata*, *A. implicata*, *A. cataracta*, *Lampsilis radiata*, *L. cariosa* and *Alasmidonta undulata*. Although all of these mussels may be found side by side buried in the muddy bottom of the lake, it is only occasionally that there is cross infection in nature. I have found small numbers of *C. curtus* associated with *C. anodontæ* in *Elliptio complanatus* and likewise a few *C. anodontæ* with *C. curtus* in *Anodonta marginata*. I have never found *C. magna* in any host but *Elliptio complanatus*. The reason for the segregation in nature is not clear, because no such condition is found in the close association of the hosts in the laboratory tanks. When various species of mussels are placed in a small aquarium cross infection results in a few days. I have examined the contents of *Anodonta* and *Elliptio* which have been kept together for a week and have found about equal numbers of *Conchophthirius anodontæ* and *C. curtus* in every specimen. There seems to be no doubt that cross infection is brought about by the ciliates leaving one host and swimming to the next, as pipette samples from the bottom of the aquarium rarely fail to reveal the presence of one or more organisms that are free from a host.

When washed free of the mantle cavity the ciliates swim about actively, but I have been able to keep them alive in a Syracuse dish for from twelve to twenty-four hours only. The movements gradually cease and death ensues although plasmolysis seldom occurs for a considerable time. Dishes containing the dead ciliates have been kept for many days with very little noticeable change in the body form.

The technical methods here employed were similar to those used in the study of *Conchophthirius mytili* (Kidder, 1933*b*), but because of the abundance of material it was found possible to fix and stain hundreds of organisms on each cover glass. The ciliates were washed into a Syracuse dish and an albuminized cover glass immersed in the fluid. After a preliminary agitation with a pipette the ciliates were allowed to settle on the cover glass which was then removed with clean forceps.

The excess fluid was drained off and the cover glass quickly immersed in the desired fixing fluid. This method proved very satisfactory as the adherent powers of these ciliates caused them to remain in close contact with the albuminized surface.

In addition to the method of Klein (1926*a, b*) for a study of the external fibrillar system, the most useful method was found to be a 4-hour fixation in strong Flemming's fluid, a thorough washing in running water and staining the long method with Heidenhain's hæmatoxylin. Differentiation was carried out in a 10 per cent solution of hydrogen peroxide. After this technique the fibers of the external and internal systems remain black while the cytoplasm is a clear yellowish gray and the nuclei and food bodies are a bluish gray. This technique has proved invaluable both in this study and in the past (Kidder, 1933*b*).

The wet silver method of Gelei and Horváth (1931) gave fairly good results in demonstrating the internal fibers of the peristomal basket and the pharynx, but I have not been able to obtain quite as sharp a differentiation of external fibers with this technique as with that of Klein or Heidenhain's hæmatoxylin destained with H_2O_2 .

For a study of the endoplasmic granules the best results were obtained by the use of the Borrel stain following Schaudinn's fluid.

For nuclear structures Heidenhain's hæmatoxylin and the Feulgen nucleic acid reaction following Schaudinn's fluid gave very good results. The former stain was also used after Bouin's, Flemming's and Champy's fluids.

Neutral red and Janus green B were used vitally but proved to be of very little value in this study.

DESCRIPTION OF SPECIES

Conchophthirius anodontæ Stein 1861

(Fig. 1)

The body outline as seen from the dorsal or ventral aspect is ovoid, being slightly more pointed at the anterior end. A lateral view shows this ciliate to be concave ventrally and slightly convex dorsally. The dorso-ventral axis is somewhat over one-third that of the antero-posterior axis. The left margin of the body forms an arc but the right side is slightly concave in the region of the buccal cavity.

The average size is $103 \mu \times 69 \mu$. The extremes of length were found to be from 125μ to 65μ , while the extremes of width were from 86μ to 47μ .

The cytostome is located on the dorsal surface in the anterior third of the body near the right margin. Anterior to the peristomal depression is an overhanging projection formed from the anterior body region.

The cytostome opens immediately under this projection and leads into a tubular pharynx which proceeds into the cytoplasm in a slightly anterior direction. The roof of the pharynx is provided with a single row of rather long cilia. The pharynx is lined with circular fibers (Fig. 1; Fig. 6*A*, *ph.*) and is contractile. It opens directly into an exceedingly long and recurved gullet (Fig. 1, *gul.*; Fig. 6, *g.*), which is a permanent path in the endoplasm and can easily be demonstrated in living and stained organisms. It is small in diameter but is quite distensible and can accommodate food bodies of considerable size. Schuberg (1889) described the intake of food but was unable to trace the gullet for any

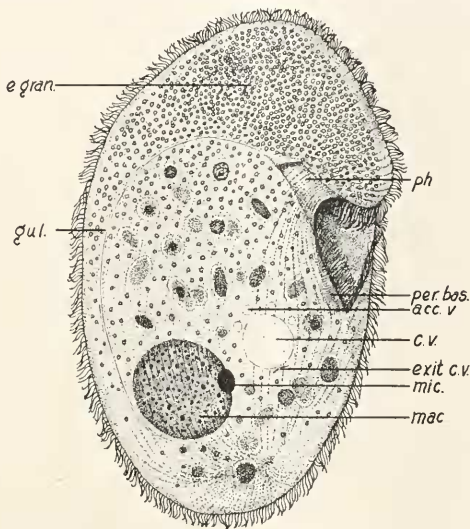


FIG. 1. *Conchophthirius anodontæ* Stein. Dorsal view. $\times 600$.

acc. v., accessory vacuoles; *c. v.*, contractile vacuole; *e. gran.*, endoplasmic granules; *exit c. v.*, aperture through which the contractile vacuole empties; *gul.*, gullet; *mac.*, macronucleus; *mic.*, micronucleus; *per. bas.*, peristomal basket; *ph.*, pharynx.

great distance. In all of my observations I have been able to demonstrate the ending of the gullet just posterior to the macronucleus (Fig. 1). The gullet sharply marks off from the rest of the body the loosely granular digestive endoplasm. I have never seen large food vacuoles outside of this region which is similar to though more extensive than the "food basket" of *Cryptochilidium bermudense* as described by Powers (1933*a*).

The peristomal field is naked and extends posteriorly from the cytostome as a rather flat, V-shaped area. It is lined with a net of fibers from which originate the ventral fibers of the peristomal basket. This basket (Fig. 1, *per. bas.*; Fig. 6, *p. b.*) is a depression between the dorsal

lip, the edge which overhangs the peristome throughout its length, and the peristomal field. The lip is supplied with a row of cilia somewhat longer than those of the body. From the longitudinal fiber at the base of this row arise the dorsal fibers which line the peristomal basket.

The cilia of the body are arranged in longitudinal rows lying in grooves. The grooves are especially noticeable in the extreme anterior portion of the body where the cytoplasm is clear. The rows originate in a wide ventral suture (Fig. 6, *E*, *v. s.*) near the anterior end, as described by Raabe (1933), and terminate in a whorl near the posterior end on the dorsal surface (Fig. 6, *E*, *d. s.*). The dorsal suture of *Conchophthirius anodontæ* is a very small area as contrasted to the extensive sutures of the other species to be described later. The rows of cilia on

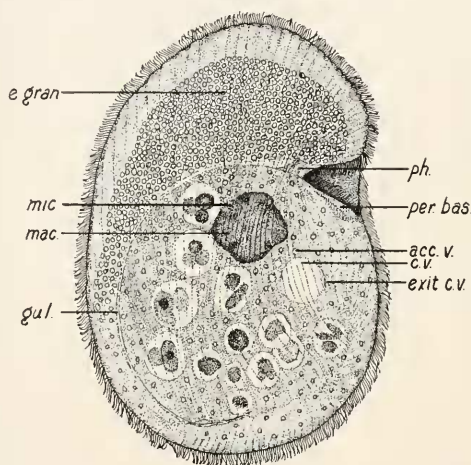


FIG. 2. *Conchophthirius curtus* Engl. Dorsal view. $\times 600$.

acc. v., accessory vacuoles; *c. v.*, contractile vacuole; *e. gran.*, endoplasmic granules; *exit c. v.*, aperture through which the contractile vacuole empties; *gul.*, gullet; *mac.*, macronucleus; *mic.*, micronucleus; *per. bas.*, peristomal basket; *ph.*, pharynx.

the ventral surface are slightly more numerous than those on the dorsal surface, while the cilia themselves are much denser on both surfaces toward the anterior end than toward the posterior.

The anterior region contains a zone of endoplasmic granules, which are quite large in this species (Fig. 1, *e. gran.*) and appear as highly refractile spheres in life. They react strongly with the indigo carmine of the Borrel mixture, appearing as a very blue shell with a less dense center. After the various silver methods this zone is quite blackened (Fig. 6, *E*). The granules are not strictly confined to this zone but are scattered sparsely throughout the rest of the endoplasm. They do not appear in the ectoplasmic layer.

The nuclei of *Conchophthirius anodontæ* are quite characteristic. The macronucleus (Fig. 1, *mac.*) is smoothly spherical and normally lies in the posterior third of the body always within the digestive endoplasm. It measures about $30\ \mu$ in diameter. It appears to be quite solid and in the resting condition is filled with spherical granules of varying size. I have never seen it distorted by adjacent food bodies to the extent observed in the two species to be described presently.

The micronucleus is very large and lies in a depression in the macronucleus (Fig. 1, *mic.*), seeming to be surrounded by the macronuclear membrane. I do not believe this to be the case, however, for at the onset of fission the micronucleus moves away from the depression, leaving it intact.

Just posterior and to the left of the peristome is to be found a single contractile vacuole (Fig. 1, *c. v.*). Varying numbers of accessory vacuoles (Fig. 1, *acc. v.*) form a ring about it and contribute to its volume as it fills during diastole. Dorsal to and in connection with the contractile vacuole is a well-defined slit-like structure (Fig. 1, *exit c. v.*) through which the contents of the vacuole empty to the outside on systole. This structure and its relationships will be discussed in detail later.

Conchophthirius anodontæ is found in great abundance in the mantle cavity, on the gills, and especially on the non-ciliated surface of the palps of *Elliptio complanatus* (Say) in 100 per cent of the molluscs examined. The hosts were taken from fresh water lakes in the vicinity of Woods Hole, Massachusetts. *C. anodontæ* is to be considered as a commensal since the food vacuoles are filled with algæ, bacteria, and sloughed-off epithelial cells of the host.

Conchophthirius curtus Engl. 1862

(Fig. 2)

In shape this species is similar to the preceding one except that the anterior and posterior ends are more bluntly truncate. The average width is proportionately greater than that of *C. anodontæ* and the average length proportionately less. The measurements of one hundred individuals gave the average length as $97\ \mu$ while the average width was $65\ \mu$. The extremes of length were from $123\ \mu$ to $61\ \mu$ and the extremes of width were from $92\ \mu$ to $51\ \mu$.

In general the peristomal region is like that of *C. anodontæ* except that the peristomal field does not extend as far posteriorly, and the pharynx (Fig. 2, *ph.*) is less conspicuous and pointed more directly toward the left margin. The gullet (Fig. 2, *gul.*) is similar but slightly more extensive than in the preceding species. The peristomal basket (Fig. 2, *per. bas.*) is smaller than in *C. anodontæ*.



The body cilia are disposed in rows, slightly closer together than those in *C. anodontæ* (compare Fig. 6, *D* and *E*), which originate from a broad ventral suture at the anterior end of the body and terminate in a long dorsal suture on the posterior dorsal surface. This dorsal suture extends nearly the width of the body and is made up of an irregular net of fibers among which are situated large basal bodies. From these basal bodies arise long cilia. My observations on this region are entirely in agreement with those of Raabe (1933) on the same species. Because of the length of the dorsal suture the ciliary rows do not come together in a whorl, as in *C. anodontæ*, but are more evenly spaced along its length.

There is a distinct difference in the appearance of the zone of endoplasmic granules in *C. curtus* and *C. anodontæ*. The granules are similar in size and staining reaction but are much more closely packed in *C. curtus*, nor does the zone extend as far out toward the anterior end of the body (Fig. 2, *c. gran.*; Fig. 6, *G, e. s.*) as in *C. anodontæ*. This difference is striking and can be noted at once even in living organisms. Engelmann (1862) called attention to this and gave it as a specific characteristic of *C. curtus*. Only a few of the endoplasmic granules are found scattered about in the middle and posterior regions.

The macronucleus of *Conchophthirius curtus* is located normally just posterior to the gullet, in the middle of the body (Fig. 2, *mac.*). It is much smaller than that of *C. anodontæ*, measuring from 18μ to 20μ in diameter. In contrast to the solid condition of the macronucleus described above, this body appears to be quite amorphous. In life it can be seen to change its shape as food bodies are pressed against it. When external pressure is applied to the organism, causing it to break, the macronucleus goes to pieces very quickly, a condition only occurring after long exposure to the external fluids in the case of *C. anodontæ*.

The micronucleus (Fig. 2, *mic.*) is extremely minute and in the resting condition is embedded in the macronucleus. At this stage I have never been able to detect it in the living organism, but only in favorably stained preparations. It can be demonstrated quite regularly after the Feulgen nucleic acid reaction as it reacts a little more intensely than the chromatin of the macronucleus and appears to be surrounded by a halo. So small is this cell element that a thorough knowledge of its structure and position was gained only after a careful study of its activity during fission. Engelmann (1862) describes the macronucleus of *C. curtus* as possessing one or two "nucleoli." I have never found more than one micronucleus in this species and I doubt very much if what Engelmann saw were micronuclei or that even one of them was a micronucleus. In life the food bodies of varying size so obscure the nuclei that in my opin-

ion no final conclusion as to the nature and number of a cell element so minute as this could be reached without recourse to careful staining techniques. Raabe (1933) figures a micronucleus in *C. curtus* almost as large as that of *C. anodontæ*, his drawing being made from *life*. Certainly the American variety of *C. curtus* will not lend itself to such simple observation.

The contractile vacuole (Fig. 2, *c. v.*) is similar in size and location to that of *C. anodontæ*. I have never found it in the posterior position described by Engelmann (1862). It is accompanied by a slitlike opening (Fig. 2, *exit c. v.*) similar to that found in *C. anodontæ*.

Conchophthirius curtus occurs in great abundance in the mantle cavity of *Anodonta marginata* Say in 100 per cent of the molluscs examined. It also occurs in fewer numbers in *Anodonta implicata* Say, *A. cataracta* Say, *Lampsilis radiata* (Say), *L. cariosa* (Say) and *Alasmidonta undulata* (Say). In nature it usually occurs alone in these mussels but may occasionally be accompanied by a very few *Conchophthirius anodontæ*. The hosts were taken from fresh water lakes in the vicinity of Woods Hole, Massachusetts, and from Lake Chautauqua, New York.

This species should also be considered as a commensal, since no indication of cell destruction of the host was noted, the food vacuoles containing algae as well as sloughed-off epithelial cells.

Conchophthirius magna sp. nov.

(Fig. 3)

The body outline of this species is oval as viewed from the dorsal or ventral surfaces, the anterior end being somewhat pointed and wider than the evenly rounded posterior extremity. The peristomal area is seen on the right margin as an indented region extending posteriorly in the form of a narrow V. When viewed from a lateral aspect, this ciliate is seen to be very much flatter than either of the preceding species.

In size it is strikingly different from *Conchophthirius anodontæ* and *C. curtus*. The average length of two hundred individuals was found to be 180 μ while the average width was 95 μ . The range of length was from 203 μ to 123 μ and the range of width from 116 μ to 63 μ .

In life *C. magna* is easily identified both by size and because it is slightly clearer and more transparent than *C. anodontæ*. Also its movements, both swimming and creeping, are less jerky and more continuous than the latter species.

The pharynx (Fig. 3, *ph.*) is shorter than the pharynx of *C. anodontæ*, but the gullet (Fig. 3, *gul.*) is extremely long and recurved toward the right side. While the gullet roughly marks off the digestive area, the food vacuoles are not so strictly limited to this region as in the

preceding species. I have never found any food bodies in the extreme anterior portion but occasionally a few are found to the left of the gullet among the endoplasmic granules. The peristomal basket (Fig. 3, *per. bas.*) is rather shallow and much longer than in *C. curtus*. The same type of structures as described for *C. anodontæ* are found in the peristomal field.

The body cilia are much finer and more closely set than the body cilia

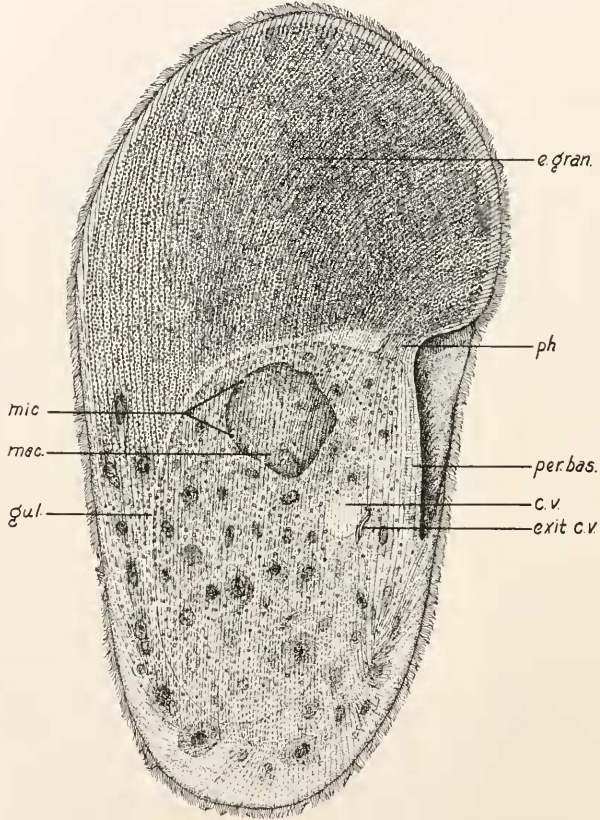


FIG. 3. *Conchophthirus magna* sp. nov. Dorsal view. $\times 600$.

c. v., contractile vacuole; *e. gran.*, endoplasmic granules; *exit c. v.*, aperture through which the contractile vacuole empties; *gul.*, gullet; *mac.*, macronucleus; *mic.*, two micronuclei; *per. bas.*, peristomal basket; *ph.*, pharynx.

of either *Conchophthirus anodontæ* or *C. curtus*. The ciliary rows are exceedingly close together on both surfaces (Figs. 6, *B* and *C*). They originate in a rather broad ventral suture (Fig. 6, *C, v. s.*) toward the anterior end and terminate in a long dorsal suture (Fig. 6, *B, d. s.*) near the posterior end. This dorsal suture is of the same type as that of

Conchophthirius curtus, although it is supplied with a greater number of large basal bodies from which long cilia originate. The region about the dorsal suture is naked except for these cilia. The longitudinal fibers of the ciliary rows proceed to the suture where their lack of basal bodies sharply defines a posterior naked zone (Fig. 6, B, p. z.). This area is much larger than in *C. curtus*.

The endoplasmic granules (Fig. 3, c. gran.) are about one-half the size of those in the preceding species. They are closely packed and fill the anterior third of the body, leaving a narrow, clear cytoplasmic layer. These granules are quite characteristic and by their relative size and extent it is possible to recognize the species immediately. A few of the endoplasmic granules are found scattered around the food vacuoles in the middle and posterior portions of the body.

The macronucleus (Fig. 3, mac.) is rather irregular in outline and is located just posterior to the gullet midway between the lateral edges. It measures about $25\ \mu$ to $30\ \mu$ in diameter. Like the macronucleus of *Conchophthirius curtus*, it is quite labile and is easily indented by the

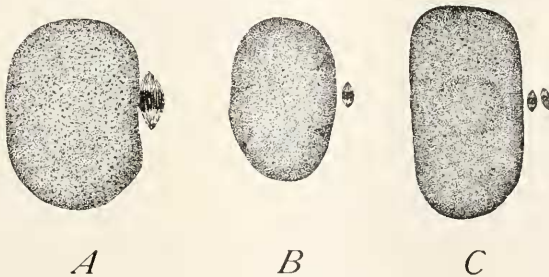


FIG. 4. The nuclear appearance during fission. $\times 900$.

A. *Conchophthirius anodontæ* Stein. The extremely large micronucleus is in full metaphase.

B. *Conchophthirius curtus* Engl. The spindle is seen to be very much smaller than in A. The macronucleus still shows signs of its folded condition.

C. *Conchophthirius magna* sp. nov. The two spindles are seen to be about the same size as the spindle of *C. curtus*.

crowding food bodies. The chromatin is dispersed in very fine granules and numerous lighter areas give the macronucleus a slightly evacuated appearance.

Embedded in the macronucleus are two minute micronuclei. These cannot be seen in the living state but can be demonstrated in the resting condition after a well-differentiated hæmatoxylin stain or after the Feulgen reaction. During fission their demonstration is most easily brought about as they then emerge from the macronucleus. Figure 4 illustrates the relative sizes and positions of the metaphase spindles of the three species here described.

The number of micronuclei in *Conchophthirius magna* appears to be not invariable. About 10 per cent of the well-prepared specimens were found to possess but one micronucleus, whereas in the remaining 90 per cent two were clearly discernible. This percentage held for the dividing organisms studied, as I shall show in a later paper.

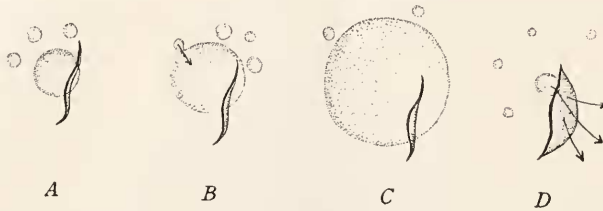


FIG. 5. Diagram representing the action of the contractile vacuole, the accessory vacuoles, and the aperture during diastole and systole.

A. Contractile vacuole slowly filling. The accessory vacuoles are large and the aperture is closed.

B. and C. Further enlargement of the contractile vacuole and fusion with the accessory vacuoles.

D. Systole of the contractile vacuole. The aperture opens and the fluid is poured to the outside. Small accessory vacuoles are again making their appearance.

The contractile vacuole (Fig. 3, *c. v.*) of *Conchophthirius magna* is similar in position though smaller in size than those of *C. anodontæ* and *C. curtus*. Like the species already described, *C. magna* possesses a slit

FIG. 6. Photomicrographs.

A. *Conchophthirius anodontæ* Stein. Ventral view of an organism fixed in strong Flemming's stained with Heidenhain's hæmatoxylin and differentiated with H_2O_2 . The photograph was taken at a mid-region focus. Note the fibers of the pharynx and peristomal basket. Also the cilia within the pharynx. $\times 630$.

B. *Conchophthirius magna* sp. nov. Dorsal view of an organism prepared by Klein's method. The anterior region of the endoplasmic granules always become very heavily impregnated with the colloidal silver. The organism is somewhat flattened. $\times 420$.

C. *Conchophthirius magna* sp. nov. Ventral view of an organism prepared as in B. to illustrate the distribution and number of ciliary rows and the ventral suture. $\times 420$.

D. *Conchophthirius curtus* Engl. Dorsal view of organism prepared as in B. Note dorsal suture at the extreme posterior end. $\times 420$.

E. *Conchophthirius anodontæ* Stein. View of an organism somewhat flattened and twisted on its left side. Preparation as in B. $\times 420$.

F. *Conchophthirius curtus* Engl. Dorsal view of a dividing organism to show the formation of the new peristomal regions. Preparation as in B. $\times 300$.

G. *Conchophthirius curtus* Engl. Ventral view of an organism fixed in Schaudinn's fluid and stained with the Borrel stain to show the zone of endoplasmic granules. $\times 420$.

d. s., dorsal suture; *c. z.*, endoplasmic granule zone; *f. b.*, fibrillar bundle; *g.*, gullet; *m.*, micronucleus; *p. b.*, peristomal basket; *p. f.*, post-oral connecting fiber; *ph.*, pharynx; *p. n.*, peristomal net; *p. r.*, pharyngeal ring; *p. z.*, posterior naked zone; *v. s.*, ventral suture.



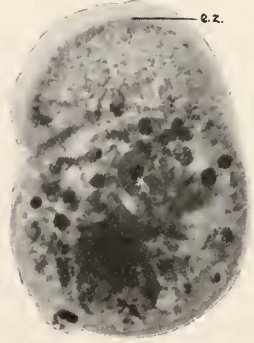
A

B



D

E



F

G

C

(Fig. 3, *exit c. v.*) through which the contents of the vacuole are emptied.

Conchophthirius magna sp. nov. was found in the mantle cavity of about one-fourth of the *Elliptio complanatus* (Dill.) examined in association with *C. anodontæ*. The infestation was never heavy, there being only from ten to twenty organisms in each host. I am a little in doubt as to its purely commensal rôle since its digestive endoplasm is always crowded with epithelial cells and I have never seen any other type of food in the vacuoles. The cells ingested may be sloughed cells but they are regular in outline and stain beautifully.

THE CONTRACTILE VACUOLE

The contractile vacuoles of the three species of *Conchophthirius* described above are identical in every respect but size. The following single description will suffice for all.

A study of the living organism under high magnification reveals the presence of a narrow slit in the dorsal body wall just posterior to the contractile vacuole. This slit is provided with a thickened margin that is highly refractile. During diastole the slit is nearly closed (Fig. 5, *A*) and as the contractile vacuole increases in size there is no apparent change. The vacuole further enlarges and absorbs the accessory vacuoles (Fig. 5, *B*) until its maximum size is reached (Fig. 5, *C*). Very suddenly the slit opens (Fig. 5, *D*), much in the manner of a purse, and the fluid contents of the vacuole pour out. The emptying is not instantaneous but takes about two seconds for completion. In this interval the details of the relationship between the slit and the vacuole can be noted. The edges of the slit are seen to be in contact with the wall of the contractile vacuole and at systole the wall appears to break through at that point.

In material stained with Heidenhain's hæmatoxylin and differentiated in H_2O_2 there is seen to be a network of fine fibers surrounding this slit. The same picture is obtained by the silver method of Klein (Fig. 8, *exit c. v.*). I have no evidence as to the nature of these fibers. That the slit is a permanent structure is demonstrated by its invariable appearance after any good method for showing fibers and also by the modification of the ciliary pattern in this region as seen in Fig. 6, *B* and *D*, and as described by Raabe (1933).

Schuberg (1889) described a tube extending from the contractile vacuole to the dorsal surface. This was also figured by Raabe (1933) and attention directed to the pore. I have never seen this in *Conchophthirius* although I have repeatedly looked for it. All specimens of this group that I have examined have this curious purse-like slit.

MacLennan (1933) has recently given a complete description of the structure of the contractile vacuoles of a number of the Ophryoscolicidæ. In these forms he finds a definite tube leading from the con-

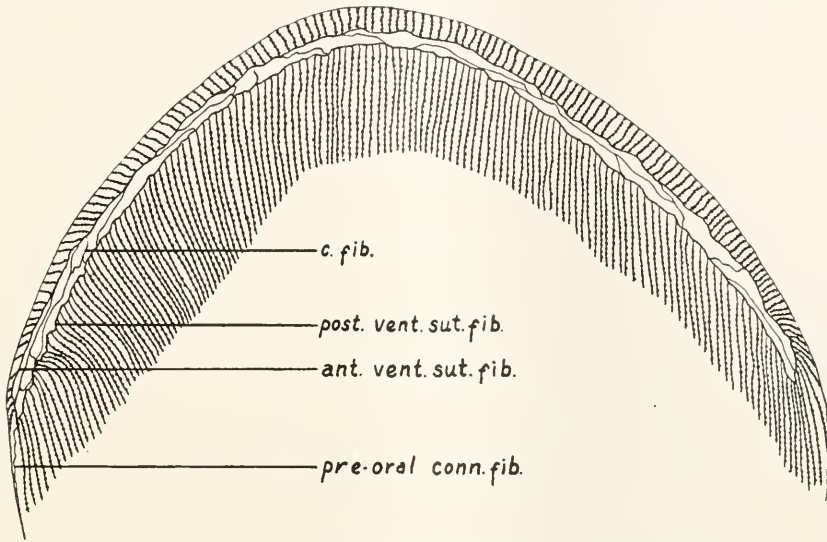


FIG. 7. Ventral view of the anterior end of *Conchophthirius magna* sp. nov. prepared by Klein's method. Note the greater number of ventral ciliary rows than dorsal. $\times 1000$.

ant. vent. sut. fib., anterior ventral suture fiber; *c. fib.*, cross fibers; *post. vent. sut. fib.*, posterior ventral suture fiber; *pre-oral conn. fib.*, pre-oral connecting fiber.

tractile vacuole through the pellicle. The pores at each end of the tube and the tube itself are permanent structures.

THE NEUROMOTOR SYSTEM

The structures of the neuromotor systems of *Conchophthirius magna*, *C. anodontæ*, and *C. curtus* are alike with the exception of minor differences of size and extent. For that reason I shall describe the system as seen in *C. magna*, calling attention to points of difference only where they occur in the other two species.

The external fibrillar system can be demonstrated clearly by the silver method of Klein or by the use of Heidenhain's hæmatoxylin differentiated with H_2O_2 . After the first method the fibers of the peristomal field are seen quite clearly but their inner connections are not shown. Since the hæmatoxylin method stains both internal and external systems, it is of great advantage to use these two methods in conjunction with one another. When one does so a wonderfully integrated system of connections is seen which I shall refer to as the neuromotor system.

The ventral suture is made up of two irregular fibers, the anterior ventral suture fiber (Fig. 7, *ant. vent. sut. fib.*) and the posterior ventral suture fiber (Fig. 7, *post. vent. sut. fib.*). From the former originate the ciliary rows of the dorsal surface and from the latter the ciliary rows of the ventral surface. These suture fibers come together at their two

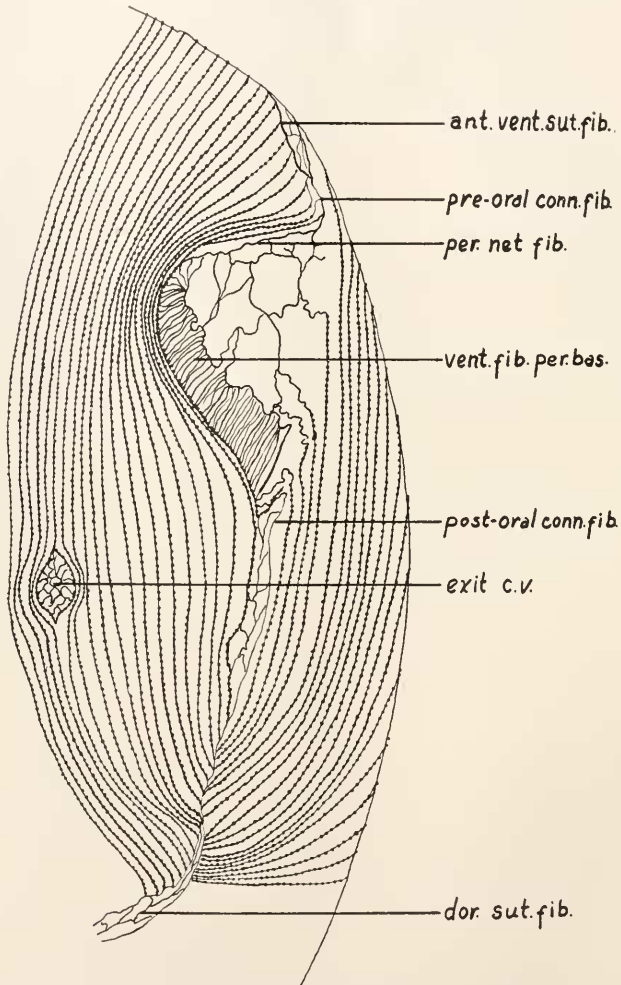


FIG. 8. Dorsal view of the peristomal region of *Conchophthirius magna* sp. nov. prepared by Klein's method. The organism is somewhat flattened. $\times 1000$.

ant. vent. sut. fib., anterior ventral suture fiber; *dor. sut. fib.*, dorsal suture fibers; *exit c. v.*, aperture through which the contractile vacuole empties; *per. net fib.*, peristomal net fiber; *post-oral conn. fib.*, post-oral connecting fiber; *pre-oral conn. fib.*, pre-oral connecting fiber; *vent. fib. per. bas.*, ventral fibers of the peristomal basket. These are probably the same as the "membranella undulans" of *Conchophthirius curtus* as described by Raabe.

ends and are also connected by short, irregularly arranged cross fibers (Fig. 7, *c. fib.*). Near the right border of the organism the joined suture fibers form a connecting fiber that curves up over the edge of the body just posterior to the projection above the cytostome. This is the pre-oral connecting fiber (Figs. 7 and 8, *pre-oral conn. fib.*; Fig. 9, *p. o.*

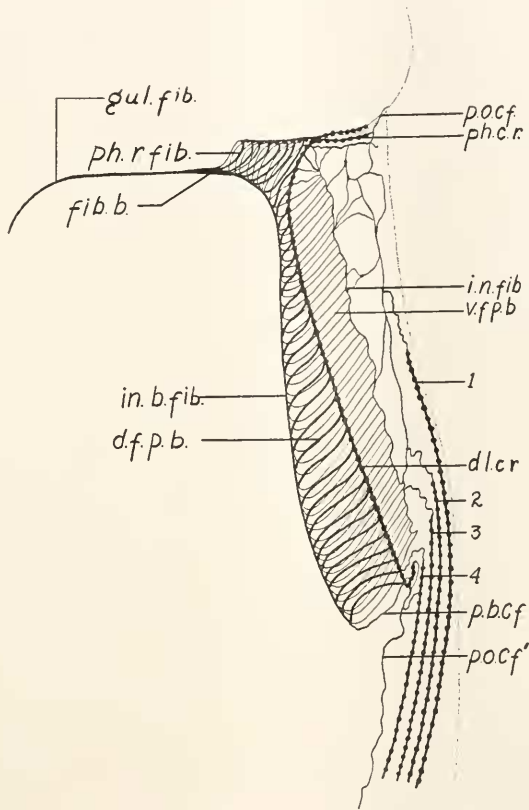


FIG. 9. Diagrammatic representation of the fibrillar system of the peristomal region of *Conchophthirius magna* sp. nov.

d. f. p. b., dorsal fibers of the peristomal basket; *d. l. c. r.*, dorsal lip ciliary row; *fib. b.*, fibrillar bundle; *gul. fib.*, fiber which follows the floor of the gullet; *in. b. fib.*, inner basket fiber; *i. n. fib.*, inner net fiber; *p. b. c. f.*, posterior basket connecting fiber; *ph. c. r.*, pharyngeal ciliary row; *ph. r. fib.*, pharyngeal ring fiber; *p. o. c. f.*, pre-oral connecting fiber; *p. o. c. f.*, post-oral connecting fiber. 1, 2, 3 and 4, four short rows of body cilia directly connected to the peristomal net.

c. f.). It gives rise to two coördinating fibers, one connecting the basal bodies of the dorsal lip ciliary row (Fig. 9, *d. l. c. r.*) and a second short fiber which connects the basal bodies of the pharyngeal ciliary row (Fig. 9, *ph. c. r.*). The pre-oral connecting fiber becomes the peristomal net fiber, which bends sharply to the left in the peristomal field (Fig. 8,

per. net fib.). From this fiber originate the secondary fibers of the network which lines the ventral floor of the peristomal field (Fig. 6, *p. n.*). These secondary net fibers, which are very irregular, are bounded on the left by a long inner net fiber (Fig. 9, *i. n. fib.*). From this numerous fine fibers arise and extend inward, lining the ventral side of the peristomal basket (Fig. 6, *A, p. b.*; Fig. 8, *vent. fib. per. bas.*; Fig. 9, *v. f. p. b.*). These are the fibers that were called by Raabe (1933) the "membranella undulans" and thought by him to originate from a row of basal bodies. This is indeed the picture one gets with the method of Klein (see Fig. 8) but it is incomplete. The ventral fibers of the peristomal basket bend dorsally and connect at right angles with a long fiber on the floor of the basket, the inner basket fiber (Fig. 9, *in. b. fib.*). From this numerous fine fibers are given off which line the dorsal surface of the peristomal basket (Fig. 9, *d. f. p. b.*) and end in the fiber of the dorsal lip.

The inner basket fiber continues anteriorly along the floor of the pharynx giving off the circular fibers of that region. At the inner end of the pharynx a large fibrous ring is found which encircles the pharynx and fuses with the inner basket fiber (Fig. 6, *A, p. r.*; Fig. 9, *fib. b.*). This fusion point is probably comparable to the motorium of *Conchophthirius mytili* (Kidder, 1933*b*) although very much smaller in size. Extending inward from the fibrillar bundle is the long gullet fiber (Fig. 9, *gul. fib.*) which continues throughout the floor of the gullet, finally fraying out at the posterior end.

The inner basket fiber is connected posteriorly with a single peristomal basket connecting fiber (Fig. 9, *p. b. c. f.*) which bends dorsally and to the right and finally connects with the dorsal connecting fiber (Fig. 6, *B, p. f.*; Fig. 9, *p. o. c. f.*). This forms the direct connection between the peristomal region and the dorsal suture. There are numerous cross fibers connecting the post-oral fiber with adjacent ciliary rows.

In *Conchophthirius magna* there are four rows of cilia that have their origin in the fibers of the peristomal net (Fig. 9, 1, 2, 3 and 4). These end in the dorsal suture. All the other ciliary rows, with the exception of those of the pharynx and dorsal lip, originate in the ventral suture, and of these all reach the dorsal suture but one, the single row that ends at the slit of the contractile vacuole (Fig. 8, *exit c. v.*).

The conditions in *Conchophthirius anodonta* and *C. curtus* differ slightly from those of *C. magna* in regard to the ciliary rows about the peristome and the fibers of the ventral and dorsal sutures. The reader is referred to Raabe's (1933) description of these fibers as they all belong to the external fibrillar system.

It will be seen from the above description that all basal bodies are intimately interconnected and also connected with the internal fibrillar system associated with the peristome, pharynx, and gullet.

During fission the old peristome disappears and two new ones are formed. The earliest stages of their formation that I was able to obtain showed a complete set of peristomal fibers, so that direct information as to the origin of these elements is lacking. Later stages (Fig. 6, *F*) show two well-marked, complete peristomes.

DISCUSSION

My measurements of *Conchophthirius anodontæ* and *C. curtus* correspond roughly to those of Raabe (1933), and to those of Engelmann (1862) for the last-named species. Engelmann's sizes for *C. anodontæ* (120 μ to 200 μ) do not agree with mine. Kahl (1931) also states that he has observed specimens of *C. anodontæ* 240 μ long. I think it highly probable that this discrepancy can be explained by the fact that such large forms measured were undoubtedly *C. magna*, as *C. anodontæ* and *C. magna* occur in the same host.

Raabe (1933) has questioned the inclusion of the genus *Conchophthirius* in the Order Trichostomida (Tribus Trichostomata of Kahl, 1931) on the grounds that these organisms possess an undulating membrane in the peristomal region and should therefore be placed in the Order Hymenostomida (Tribus Hymenostomata of Kahl). I have already pointed out that Raabe must have mistaken the fibers of the peristomal basket for an undulating membrane. When observed after the method of Klein these fibers might well be interpreted as cilia arising from the basal bodies of the dorsal lip (see Fig. 8).

The endoplasmic granules described above are so characteristic and so persistent that one is tempted to speculate on their function. While it is perhaps conceivable that they represent secretory granules comparable to those described by Powers (1933*b*) in *Entodiscus borealis*, at present I have no evidence for such an assumption. A discussion of function, therefore, must wait until a future date.

The rhythmic opening and shutting of the purse-like slit dorsal to the contractile vacuole seems to demand a structural explanation. The presence of a network of fibers in this region suggests that they may be of the nature of myonemes, thereby accounting for the action of the slit. It has been tacitly assumed by the majority of protozoölogists that the action of the contractile vacuole was the result of osmotic pressure and that no protoplasmic differentiation was necessary. Ray (1932), however, has described internal fibers about the contractile vacuoles of *Balantidium sushilii*, but he does not state the supposed

function of these fibers. I have never found such fibers in the region of the contractile vacuole in *Conchophthirius* but only fine fibers about the slit. Because of the direct connection of these fibers about the slit to the longitudinal fibers of the ciliary rows, I regard them as a part of the external fibrillar system, perhaps functioning as contractile or conductive elements or both.

The concept of the neuromotor system has been discussed by many in the past and more recently by MacLennan and Connell (1931), Powers (1933b), Kidder (1933b), Turner (1933) and Lund (1933). Lund has given a complete review of the literature dealing with the fibrillar system of *Paramecium* and has correlated the external system (silver-line system) with the internal fibrillar system. Turner has demonstrated a similar connection between the two systems in *Euplotes patella*. As has been pointed out by these workers, the conclusion of the American investigators as to the nature and function of these various fibers is largely in agreement. The acceptance of a system of coordinating fibers, functioning as impulse transmitters, seems to be inevitable. This does not exclude the possibility, as mentioned by Powers (1933b), that elements connected with this system may have other functions as well, such as supportive and contractile. Indeed, Powers suggests just such a triple function for the "stomatostyle" of *Entodiscus borealis*.

My observations on the various species of *Conchophthirius* have led me to agree entirely with the above conclusions. Also I must point out that the possibility of a set of fibers functioning both as conductile and contractile elements seems to be strengthened by a study of members of this genus. The fibers encircling the pharynx are directly connected with the various peristomal fibers and also with the coordinating fibers of the pharyngeal ciliary row, yet the pharynx is capable of complete contraction. The contraction occurs when food bodies are swept in and by this action the food is forced into the gullet. It seems logical to suppose that the local contraction of the pharynx is brought about by the pharyngeal fibers. The fibers surrounding the slit above the contractile vacuole may be supposed likewise to possess a dual function. It seems possible that in addition to the functions of contractility and conductivity these two sets of fibers may lend some support to their two areas which are subjected to the greatest strains.

No large body could be demonstrated, in the three species described above, that would correspond to the motorium of *Conchophthirius mytili* (Kidder, 1933b). This is not a serious issue, and in no way impairs the concept of a neuromotor system. Turner (1933), after carefully re-investigating the fibrillar system in *Euplotes patella*, came to the conclusion that the motorium of Yocom (1918) was only a pellicular fold

and that the fibers joined one another directly. The situation described above corresponds to these findings. In the position occupied by the motorium of *Conchophthirius mytili* (under the pharynx), the fibers of *C. anodontæ*, *C. curtus*, and *C. magna* join in a bundle and instead of having a massive structure only the thickening due to the fusion of fibers is seen. This bundle is the only concentration of material that could be interpreted as a coördinating center, and I see no objection to the idea that it may function as such.

The specific differences that exist between the members of the genus *Conchophthirius* from fresh water mussels involve the pattern of the ciliary rows, the position and extent of the posterior dorsal suture, the position, shape, and number of the nuclei and to some extent the size. In no case is the difference so profound as between *Conchophthirius mytili*, *C. steenstrupii* or *C. caryoclada* where the relationship of the position of the cytostome to the rest of the body varies greatly. It may be found advisable, when more comparative observations have been made, to subdivide the genus, restricting the term *Conchophthirius* to those forms with the general structural relationships possessed by the type species *Conchophthirius anodontæ*.

SUMMARY

1. Three species of the genus *Conchophthirius* Stein are described: (1) *C. anodontæ* (Ehr.) Stein, from the mantle cavity, palps, and gills of *Elliptio complanatus* (Dill.); (2) *C. curtus* Engelmann, from the mantle cavity of *Anodonta marginata* Say, *A. implicata* Say, *A. cataracta* Say, *Lampsilis radiata* (Say), *L. cariosa* (Say), and *Alasmidonta undulata* (Say); and (3) *C. magna* sp. nov. from the mantle cavity of *Elliptio complanatus* (Dill.).

2. The chief structural differences between the three species are noted in the shape, size, and extent of the peristome, distribution and size of the endoplasmic granules, distribution and number of the ciliary rows, type of posterior dorsal suture, and shape, size, and number of nuclei.

3. The host specificity seems to be marked in the case of *Conchophthirius magna*, less marked in the other two species.

4. Cross infection undoubtedly takes place by swimming trophozoites.

5. A peculiar permanent slit through which the contractile vacuole discharges is described.

6. A well integrated and closely interconnected neuromotor system is described, consisting of external, internal, and peristomal fibrillar systems.

7. The neuromotor system is thought to be mainly conductile but some parts of it may possibly be contractile or even supportive.

LITERATURE CITED

- ANDRÉ, EMILE, 1910. Sur quelques infusoires marins parasites et commensaux. *Revue Suisse Zool.*, **18**: 173.
- CERTES, A., 1891. Note sur deux infusoires nouveaux des environs de Paris. *Mém. Soc. Zool. France*, **4**: 536.
- CLAPARÈDE, E., AND J. LACHMANN, 1858. Études sur les infusoires et les rhizopodes. Geneva.
- DE MORGAN, W., 1925. Some Marine Ciliates in the Laboratory Tanks at Plymouth, with a Description of a New Species, *Halophrya coronata*. *Jour. Marine Biol. Assoc. of United Kingdom*, **13** (N. S.): 600.
- ENGELMANN, T. W., 1862. Zur Naturgeschichte der Infusionsthier. *Zeitschr. f. wiss. Zool.*, **11**: 347.
- GELEI, J. VON, AND P. HORVÁTH, 1931. Eine nasse Silber-bzw. Goldmethode für die Herstellung der reizleitenden Elemente bei den Ciliaten. *Zeitschr. f. wiss. mikrosk. u. f. mikrosk. Tech.*, **48**: 9.
- GHOSH, EKENDRANATH, 1918. XV. Studies on Infusoria. II. Two new species of Conchophthirus, Stein. *Records of the Indian Museum*, **15**: 131.
- KAHL, A., 1931. Die Tierwelt Deutschlands. 21 Teil: Protozoa, p. 287. Fischer, Jena.
- KIDDER, GEORGE W., 1933a. *Conchophthirus caryoclada* sp. nov. (Protozoa, Ciliata). *Biol. Bull.*, **65**: 175.
- KIDDER, GEORGE W., 1933b. Studies on *Conchophthirus mytili* de Morgan. I. Morphology and division. *Arch. für Protist.*, **79**: 1.
- KLEIN, BRUNO M., 1926a. Über eine neue Eigentümlichkeit der Pellicula von *Chilodon uncinatus* Ehrbg. *Zool. Anz.*, **67**: 160.
- KLEIN, BRUNO M., 1926b. Ergebnisse mit einer Silbermethode bei Ciliaten. *Arch. für Protist.*, **56**: 243.
- LUND, EVERETT E., 1933. A Correlation of the Silverline and Neuromotor Systems of *Paramecium*. *Univ. Calif. Publ. Zoöl.*, **39**: 35.
- MACLENNAN, R. F., 1933. The Pulsatory Cycle of the Contractile Vacuoles in the Ophryoscolecidae, Ciliates from the Stomach of Cattle. *Univ. Calif. Publ. Zoöl.*, **39**: 205.
- MACLENNAN, R. F., AND F. H. CONNELL, 1931. The Morphology of *Eupoterion pernix* gen. nov., sp. nov. *Univ. Calif. Publ. Zoöl.*, **36**: 141.
- MERMOD, GASTON, 1914. Recherches sur la faune infusorienne des tourbières et des eaux voisines de Sainte-Croix (Jura vaudois). *Revue Suisse Zool.*, **22**: 82.
- POWERS, PHILIP B. A., 1933a. Studies on the Ciliates from Sea Urchins. I. General taxonomy. *Biol. Bull.*, **65**: 106.
- POWERS, PHILIP B. A., 1933b. Studies on the Ciliates from Sea Urchins. II. *Entodiscus borealis* (Hentschel), (Protozoa, Ciliata), behavior and morphology. *Biol. Bull.*, **65**: 122.
- RAABE, ZDZ., 1933. Untersuchungen an einigen Arten des Genus *Conchophthirus* Stein. *Bull. Acad. Polonaise des Sci. et Lettres. Série B: Sci. Nat.* (II): 295.
- RAY, HARENDRANATH, 1932. On the Morphology of *Balantidium sushilii* n. sp., from *Rana tigrina* Daud. *Jour. Roy. Mic. Soc.*, **52**: 374.
- SCHUBERG, AUGUST, 1889. Die Gattung *Conchophthirus* Stein. *Arbeit. a. d. Zool.-Zootom. inst. Würzburg.*, **9**: 65.
- STEIN, F., 1861. Ueber ein parasitisches Infusionsthier aus dem Darmcanal von Paludinen. in: *Sitzungsber. k. bohm. Ges. d. Wiss. zu Prag.*, p. 85.
- TURNER, JOHN P., 1933. The External Fibrillar System of *Euplotes* with Notes on the Neuromotor Apparatus. *Biol. Bull.*, **64**: 53.
- YOCOM, H. B., 1918. The Neuromotor Apparatus of *Euplotes patella*. *Univ. Calif. Publ. Zoöl.*, **18**: 337.